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A CYTOLOGICAL STUDY OF YEAST *(SACCHAROMYCES CEREVISIAE)*[1]

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INTRODUCTION

A century ago Nägeli (1844) first described the yeast cell as having "a little nucleus of whitish mucus, lying on the membrane." From that time to this the cytology of yeast has been a subject of controversy. Extensive bibliographies and discussions of the earlier literature may be found in Wager ('98), Wager and Peniston ('10), Guilliermond ('20), Moore ('33), and Badian ('37). Brandt ('41) has reviewed the more recent literature, as well as given the results and interpretations of his own extensive investigations. Recently Lindegren ('45) and Subramaniam and Ranganathan ('45, '46a, '46b) have published differing interpretations of nuclear organization in yeast.

In spite of the great amount of work which has been done on the cytology of yeasts, there is still no general agreement (Table VI) among students of the subject even on fundamental points. There are several reasons for this lack of agreement: (1) The most important is the small size of the yeast cell which makes accurate observation of details extremely difficult and causes interpretation to be more or less speculative. (2) The use of only one or two stain techniques by many investigators has increased the confusion because interpretations are based on incomplete information. (3) Until the work of Winge and his associates ('35, '37) investigators were handicapped by lack of understanding of the life cycle of yeasts. (4) Not until the recent work of Lindegren and Lindegren ('44) was it possible for a cytologist to secure adequate sporulating material.

The present work was undertaken with the hope that additional information could be gained concerning the structure of the yeast cell through the thorough and methodical use of a variety of techniques on the excellent material available from the Lindegren laboratory.

[1]An investigation carried out in the graduate laboratory of the Henry Shaw School of Botany of Washington University, assisted by a grant from Anheuser-Busch Inc., and submitted as a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Material

Saccharomyces cerevisiae was used in all of the work reported in this paper except as noted below. The cultures were secured from the Lindegrens, and all culture designations refer to their material. Most work was done with the diploids LK, with the hybrids EFF, 30 x 20 cc., and with BC20—, and several similar haploids. EFF is a hybrid of three commercial baking yeasts; LK is isolated from a Canadian pressed yeast. The haploid BC20— is a single ascospore isolate from a strain studied by the Lindegrens as were the other haploids used.

While working on the identification of wild yeasts found on native fruits obtained from the Missouri Botanical Garden Arboretum, an especially large one which showed peculiar multiple budding was isolated. It is probably an undescribed species of *Syringospora* or a related genus. Limited cytological observations were made on it for comparison with *Saccharomyces cerevisiae*. A few observations were also made on a species of *Cryptococcus* (?), another wild yeast.

Methods

In so far as possible all techniques and observations were carried out with all material used; that is, a general systematic survey was attempted. Parallel observations of living material were also made.

Culture Methods.—Budding cells of various diploid cultures were sometimes obtained from yield tests in the Lindegren Laboratory (Lindegren and Lindegren, '43). Both haploids and diploids were also cultured in liquid nutrient medium

TABLE I

PROCEDURE FOR SECURING SPORULATION (LINDEGREN AND LINDEGREN, '44)

Time	Medium			Technique
	Kind	Formula	Method of Preparation	
48–72 hrs.	Presporulating	Beet (leaves) extract ....10 cc. Beet (roots) extract......20 cc. Apricot juice ....35 cc. Grape juice ....16½ cc. Yeast (dried) ....2 gm. Glycerin ....2½ gm. Agar ....3 gm. $CaCO_3$ ....1 gm. Water—to give 100 cc. final volume	Steam mixture 10 minutes. Tube and autoclave. Slant.	Streak on slant; incubate at 25° C. (room temp.). After 2–3 days pour 1 cc. sterile water over yeast. Stir to make thick suspension of cells. Take up in sterile pipette.
16–48 hrs.	Gypsum slant	Water ....100 cc. Plaster of Paris ....100 gm.	Mix, pour, and slant rapidly. Dry 24 hours at 50° C. Autoclave.	Pour over upper part of gypsum slant. Pipette 3–4 cc. sterile water containing enough acetic acid to bring it to pH 4.0 into lower half of slant. Incubate at 25° C.

and on nutrient agar slants. Little work was done with copulating yeast cells or with old or starved cultures.

Material for the study of stages of sporulation was cultured in accordance with the method developed by the Lindegrens on medium supplied by them (Table I). The diploid strains LK and EFF were usually used because they sporulate well.

Smear Technique.—All permanent preparations were made as smears on cover glasses and stained in cover glass Coplin jars (cover glass wells of Thomas & Co.). The yeast cells were stuck to the cover glass with a thin coating of Mayer's albumen fixative or applied directly to a thoroughly cleaned cover glass. The cover glasses with the layer of albumen were generally heated gently over an alcohol burner "until a gray smoke is given off" as suggested by La Cour ('41) before applying the cells. Many more cells remained on the cover glass if fixative was applied, but with some techniques the albumen tended to stain and if drying occurred artifacts frequently appeared. Many cells were lost in succeeding operations if applied directly to the cover glass.

For applying the yeast to the cover slip a micropipette was found useful. An ordinary glass dropper was heated just above the narrow end, drawn out thin, cut off to a length of seven or eight inches, and the rubber bulb replaced. A very small drop of water was placed on the cover glass with the micropipette and cells were added from a slant with a sterile loop; in other cases a droplet of cells was pipetted onto the cover slip from a suspension. The cells were spread evenly by passing the thin flexible end of the micropipette across the surface of the cover glass. This was done rapidly to avoid drying, which causes severe distortion of the cells. The cover glasses were immediately floated cell-side down on the surface of the fixing solution in a Petri dish. If long fixation was required, they were transferred after about fifteen minutes to a cover glass Coplin jar for convenient storage.

Fixation and Staining Procedures.—Many different fixations were tried, and the types which apparently caused least shrinkage, distortion of the vacuole, and granulation of the cytoplasm were generally used (Table II). Drying, even after fixation, tended to cause shrinkage and distortion. Good fixation was apparently related in part to the condition of the cells; fresh, actively growing cells showed better fixation than old cells from giant colonies, and actively budding cells better than the highly granular sporulating cells. None of the methods used was completely satisfactory.

The general staining procedures are outlined in Table III. A further discussion of outstanding points is given in the following section of the paper.

TABLE II

FIXATION*

Fixative	Formula and/or Modifications	Time	Evaluation
Mercuric chloride	Saturated aqueous $HgCl_2 + 0.5 - 1.0\%$ glacial acetic acid	½–24 hrs.	Most satisfactory general fixation
Iodine-formalin-acetic acid	1% iodine in 1% KI..20.0 cc. Formalin 40% .... 4.0 cc. Glacial acetic acid ...... 0.5 cc. Distilled water ....5–10.0 cc.	12–24 hrs.	Good with Giemsa technique (2, Table III)
Navashin	Plant tissue formula (Lee, '37) "Craf" Modification (Johansen, '40)	1–12 hrs.	Fair. Distortion of vacuole common
Osmic acid vapor	Vapor from 1% osmic acid solution	3–5 min.	Fair. Cytoplasm granular, vacuole often distorted or invisible
Picric acid	Saturated in distilled water or 70% alcohol	½–12 hrs.	Fair with methylene blue-eosin technique (7, Table III)

*Other fixations tried but not used because of distortion, shrinkage, and/or granulation of the cytoplasm were Carnoy, Carnoy-Lebrun, Perenyi, Flemming's weak solution, Uranium fixative (Semmens, '42), Picro-formol-acetic acid mixtures, glacial acetic acid vapor, chrome-Bowen-urea fluid, and alcohol both alone and in various combinations.

TABLE III

STAINING METHODS EMPLOYED IN THE STUDY OF THE YEAST CELL*

Stain	Usual fixation	Staining procedure	Remarks
1. Feulgen (Johansen, '40; de Tomasi, '36; Coleman, '38)	Mercuric chloride Navashin (Unnecessary to wash out fixing solution before hydrolysis—Bensley & Bensley, '38)	1. Hydrolyze 7–8 min, in 1 *N* HCl at 60° C. (Hillary, '39). 2. Stain 4–5 hrs. in Feulgen. 3. Wash 10 min. in each of three $HCl\text{-}K_2S_2O_2$ baths.	Feulgen positive body present in all cells. Cytoplasm clear and colorless.
2. Robinow's bacterial Giemsa (Robinow, '42; Dubos, '45)	Mercuric chloride Iodine Osmic vapor	1. Hydrolyze in 1 *N* HCl at 60° C. 7–10 min. 2. Stain in Giemsa (1 drop stain per ml. dilute buffer— pH 6.9–7.0) 15–45 min. 3. Pass through acetone-xylol mixtures as follows: 20:1 .... 4–30 sec. 14:6 ....10–30 sec. 6:14 ....10–15 sec. Pure xylol ....10–15 min.	Very good; apparently stains Feulgen positive body and gives stronger contrast and differentiation.

TABLE III (continued)

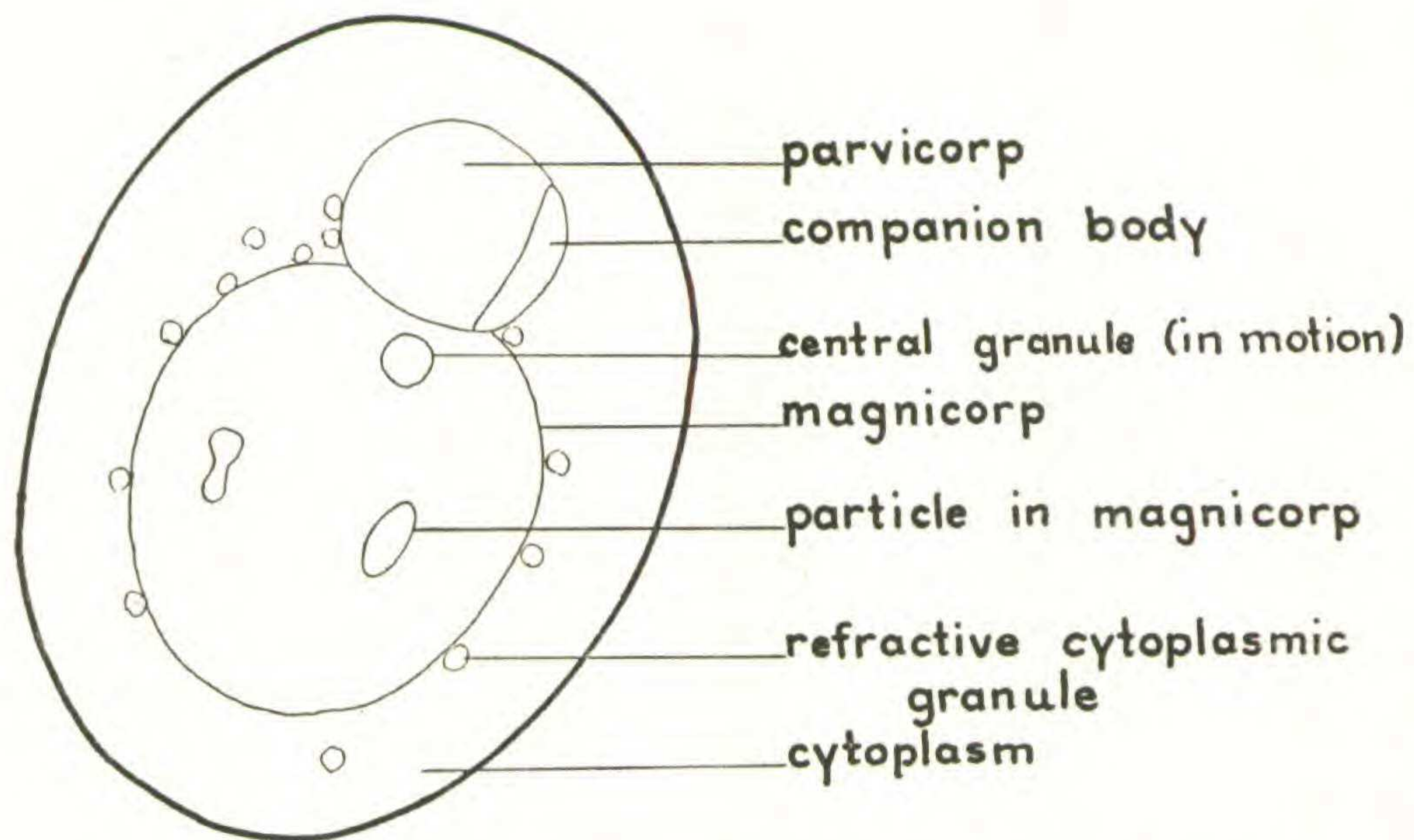
3. Methyl violet (Johansen, '40)	Navashin	1. Stain in 1% aqueous methyl violet 10 min. 2. Pass through: a. 0.5% picric acid in 70% alcohol 10 sec. b. 0.5% picric acid in 95% alcohol 10 sec. c. 95% alcohol (plus 4 drops NH_4OH per 100 cc.) 15 sec. d. 100% alcohol 10–15 sec. e. Clove oil 30–45 sec.	Results only fair; nothing added to those from other techniques.
4. Flemming's triple stain (Johansen, '40)	Navashin	1. Mordant 24 hrs. in 1% osmic acid in 2% chromic. 2. Stain in standard safranin solution 24 hrs. 3. Stain in 1% aqueous crystal violet 10–15 min. 4. Dip twice in 95% alcohol, 3 or 4 times in absolute. 5. Drop sat. sol. orange G in clove oil on c. g. 10 sec. 6. Wash in clove oil.	Results poor; added nothing to hematoxylin; could probably be improved by modification.
5. Heidenhain's hematoxylin (Johansen, '40; Lee, '37)	Mercuric chloride Navashin	1. Mordant in 4% ferric ammonium sulfate. Procedure a. 6–18 hrs. Procedure b. 1 hr. 2. Stain in 0.5% hematoxylin. a. 6–8 hrs. or overnight. b. 2–3 hrs. 3. Destain in sat. aqueous picric acid (Tuan, '30).	Shorter mordanting, staining, and destaining gave different picture from long. Cytoplasm tends to stain.
6. Brazilin	Same as Heidenhain's hematoxylin	1. Mordant 6 hrs. in 1% ferric ammonium sulfate in 70% alcohol. 2. Stain 6 hrs. in 0.5% Brazilin in 70% alcohol. 3. Destain in sat. picric in 70% alcohol.	Results similar to Heidenhain's hematoxylin.
7. Methylene blue-eosin (Badian, '37)	Picric acid Navashin Mercuric chloride	1. Overstain in 1% aqueous methylene blue 24–48 hrs. 2. Differentiate very slowly with dilute eosin (0.5% or less).	Results uncertain. Tends to lose stain in alcohol.
8. Aceto-, lacto-, and propionic carmine, orcein, Bismark brown	Alcohol-acetic acid. Also various mordants and pretreatments were used without success.	All combinations of the acids and stains in various concentrations were tried—heated and unheated, fresh and after standing. No permanent slides prepared.	Generally unsatisfactory. Aceto-orcein and propionic carmine usually some better than other combinations.

*All preparations were run up through alcohol series into xylol and mounted in balsam unless otherwise stated. Usual washing or rinsing steps not listed.

OBSERVATIONS

INTRODUCTION AND TERMINOLOGY

As mentioned in the Introduction there is as yet no general agreement among yeast cytologists even as to fundamentals. The body most frequently referred to as the nucleus (Guilliermond, '10, '20; Badian, '37; Beams, Zell, and Sulkin, '40) is called the centriole by another school (Lindegren, '45) and the nucleolus by a third (Wager and Peniston, '10). In the following observations new terms which are purely descriptive and have no previous connotation have accordingly been chosen in order to present a picture of results, independent of interpretations of nuclear organization in yeast. The term "parvicorp" (small body) will be used (text-fig. 1) to indicate that part of the yeast cell frequently designated as the



Text-fig. 1. Diagram of the yeast cell with parts labeled according to the terminology used in this paper.

nucleus. The term "magnicorp" (large body) will be used to designate the part usually called the vacuole, but also named the nucleus (Janssens and Leblanc, '98) and the nuclear vacuole (Wager and Peniston, '10; Lindegren, '45). A diagrammatic representation of these parts is shown in text-fig. 1.

Descriptions of budding material precede those of sporulating material. The results for each technique are given separately and in the order listed in Table III. The drawings on the plates follow the observations of the stained slides as closely as possible. All observations were made with a binocular microscope equipped with a 90 x apochromatic objective and 15 x compensating oculars.

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Studies of Budding Cultures

Feulgen Technique.—In all cells except small buds the parvicorp is at least faintly Feulgen positive (pl. 10). However, not all of the parvicorps exhibit the same color depth; a few are small and stain much more strongly than the larger ones. In some cells the parvicorp is evenly colored throughout, in others there is apparent variation in color intensity (pl. 10, figs. 4, 5, 11, 13). The buds often lack the parvicorp, but in that case it is frequently dividing in the mother cell or has finished its division or may not have started to divide, in which event it is commonly larger than usual (pl. 10, figs. 1–15). The division may be completed in the mother cell and the parvicorp then pass into the bud, but in many strains it more often appears to divide directly into the bud. In actively growing cultures the parvicorp is usually located proximal to the small bud and divides directly into it (Lindegren, '45). Where the budding is not quite so rapid the Feulgen positive body may be located between two parts of the magnicorp or even opposite the bud. After the buds acquire parvicorps the latter usually assume a distal position in mother and daughter cells (pl. 10, fig. 16). The parvicorp may vary in shape from round to oval, may be crescent-shaped, irregular in outline, or divided into two bodies, as noted earlier by Margolena ('32), Winge ('35), Badian ('37), and others. These bodies have often been interpreted as chromosomes (Table VI). Conventional mitotic figures were not seen in the dividing Feulgen-stained bodies of *Saccharomyces cerevisiae.* The Feulgen stain is faint and the parvicorp small, so that details are somewhat speculative.

The magnicorp, which was unstained but generally quite clear in outline, showed no Feulgen positive material with the procedure used. A discussion of the magnicorp will be deferred to the sections on methylene blue-eosin and Heidenhain's hematoxylin in which its structure is more readily observed. However, it might be mentioned here that the parvicorp almost universally lies in contact with the magnicorp.

Robinow's Giemsa Technique.—The use of this technique was suggested by La Cour (personal communication) after a number of others were found inadequate. The Robinow Giemsa bacterial stain ('42; Dubos, '45, with appendix by Robinow) was used with the following minor modifications. Osmic acid was slightly less satisfactory than mercuric chloride or iodine-formol-acetic acid as a fixation. Samples of stain were obtained from three sources and were found to vary somewhat in their staining ability. Destaining was not always even. Insufficiently destained and over-destained cells sometimes occurred on the same slide probably due to inadequate pH control. Difficulty with fading was overcome by controlling the pH of the various solutions, especially the balsam which was neutralized with sodium bicarbonate following the directions in Lee (9th edition, '28) and by storing the finished slides in darkness. The better slides produced by this method were the most satisfactory for observation of the parvicorp. However, good results were obtained only with the correct balance of all variables.

Giemsa stains the parvicorp in much the same manner as the Feulgen reaction but gives a much deeper color that shows more internal differentiation and greater contrast with the cytoplasm. Because of this sharpness of contrast the irregularity of the outline of many of the parvicorps is much more apparent than with Feulgen. Although many of the cells show parvicorps with relatively regular outlines more of those with irregular outlines are illustrated in pl. 11 in order to indicate the kind and degree of variation observed. The difference in color intensity within the stained bodies was often quite marked, resembling somewhat the chromocenters of higher plants. One or two small extrusions or appendages were often found. In older agar slant cultures, apparently aberrant divisions of the parvicorps without consequent division of the cell seemed to occur, giving such forms as shown in pl. 13, figs. 92, 93, 95–97. Haploid cells and their parvicorps were smaller than diploid, but measurements of the parvicorps were not attempted because their size variation in different stages of cell division made exact comparisons of doubtful value. Division of the parvicorp appeared much the same as with the Feulgen technique. A few of the parvicorps exhibited the bipartite structure noted in the preceding technique but the phenomenon seemed less in evidence, especially in the haploids.

The magnicorp was generally not visible with this technique, but when it was its relation with the parvicorp was usually clear and unquestionable. In these cells it lay in direct contact with the parvicorp as described above (pl. 11, figs. 30, 40, 42, 46). In a few cases various other relationships between these two bodies were observed, and several of these are illustrated (pl. 11, figs. 45, 47; pl. 13, figs. 96, 97). In these exceptional cells, however, the definition of the two bodies was somewhat obscure.

Methyl Violet and Flemming's Triple Stain.—Johansen's methyl violet staining method was used without appreciable change. Methods developed by Smith ('34), Newton (Johansen, '40), and Hancock ('42) did not give good results but would probably prove satisfactory if time were taken to modify them.

The parvicorp generally stained in much the same manner as with Feulgen (pl. 12, figs. 48–60), but sometimes only part of it retained the stain, the slides thus resembling certain Heidenhain's hematoxylin slides (pl. 12, figs. 51, 53, 56). The magnicorp showed no particles or stained structures. Similar but less certain results were obtained with Flemming's triple stain (pl. 12, figs. 61, 62). As these staining procedures provided little additional information to that acquired from other techniques and as they were more troublesome to carry out, they were not used extensively.

Heidenhain's Hematoxylin and Brazilin.—After long mordanting and staining with Heidenhain's hematoxylin (5a, Table III) the entire parvicorp remained black (pl. 13, figs. 72–81). With the short staining procedure (5b, Table III) usually a black area designated here as the companion body (text-fig. 1) stained at one place and the remainder of the parvicorp was lighter than the surrounding cyto-

plasm (pl. 13, figs. 82–89). This differentiation resembled that shown in Wager and Peniston's figures of the "chromatin patch" except that the "peripheral layer of chromatin" was not generally apparent, nor did the companion body exhibit as great a diversity of shape as the authors illustrate (Wager and Peniston, '10, pl. 16, figs. 53–80). This same phenomenon shows to some extent with Brazilin and methyl violet techniques but is not apparent with any of the others that were used, although identical fixations were frequently employed.

In either budding or resting cells this companion body was often double and occurred in any one of several positions, probably due to both the orientation under the microscope and the condition and stage of development of the cell. Division of the companion body at mitosis is difficult to follow even in slides of actively budding cultures. It seems to elongate greatly and divide by thinning in the center (pl. 13, figs. 83, 89). In the non-budding cell the parvicorp appears to have a very regular outline; in the budding cell it is not distinct as the contrast between cytoplasm and parvicorp is not great. With the longer staining procedure results are similar to those of the Feulgen and Giemsa techniques, but no differentiation within the parvicorp is visible, the boundary is usually regular, and no extrusions or appendages are apparent. The parvicorp may destain in various unusual patterns, especially in old or poorly nourished cells, and give rise to figures such as pl. 13, fig. 76, which, although they may resemble conventional mitotic stages, probably do not represent a division stage at all. Because the cytoplasm sometimes destains irregularly, results are difficult to interpret. Therefore, in spite of the recent work of Subramaniam and Ranganathan ('45), the author does not consider this technique one of the most satisfactory for the study of yeast cytology, certainly not the technique to use as the sole basis of interpretation.

The magnicorp appears clear and optically empty in budding material. However, if it is shrunken or distorted in fixation, as sometimes happens, the folds tend to retain the stain and give the appearance of strands passing over the surface (pl. 13, fig. 73). In well-expanded and preserved magnicorps this phenomenon was not apparent. Wager and Peniston reported a "chromatin network" over the surface of the magnicorp as a regular cell component, possibly because they regularly dried their preparations after fixation. There are times when denser strands of cytoplasm seem to radiate from the parvicorp and may or may not extend out over the magnicorp depending upon the relative positions of the two cell bodies (pl. 13, figs. 77, 78). The early entrance of the magnicorp into the bud is especially apparent with this stain when it follows mercuric chloride fixation. Subramaniam and Ranganathan have used a Carnoy fixing solution which usually leaves the magnicorp invisible and the relative positions of cell components uncertain.

Brazilin gives results similar to Heidenhain's hematoxylin, staining either the entire parvicorp or only the companion body depending upon the length of staining and destaining (pl. 12, figs. 63–71). With the shorter procedure the parvi-

corp sometimes shows shadowy material extending from the companion body (pl. 12, fig. 65).

Methylene Blue-Eosin Technique.—The Giemsa-eosin technique of Badian ('37) was modified by substituting methylene blue for Giemsa. It can be said of it also: "L'application de cette méthode de différenciation aux Levures n'est pas toujours facile—" [Badian, '37, p. 64]. Staining and differentiation of the cells in bulk in a centrifuge tube were more easily controlled than on the cover glasses although both methods were used.

Certain particles or granules in the yeast cell stained a brilliant blue-red when the differentiation was carried just far enough; further differentiation in eosin left the entire cell a faint, even pink. The position, number, and size of these particles apparently depended in part on physiological conditions, in part on fixation. In a 48-hour unshaken broth culture the stained bodies, if present, were found within the magnicorp (pl. 14, figs. 98–107); in a similar culture that was shaken and crowded colored particles appeared to lie at the periphery of the magnicorp or occasionally in the cytoplasm (pl. 14, figs. 109–112). In the unshaken culture, the parvicorp stained faint pink in the blue cytoplasm and was clearly visible in 98 per cent of the cells, but little or no differentiation was noted. The outline was quite regular and clear. In this same culture approximately 85 per cent of the yeast cells contained no particles at all in the magnicorp; the rest contained one or more of various sizes and shapes (Table IV). In the shaken culture the

TABLE IV

NUMBER OF PARTICLES PER CELL IN THE MAGNICORP OF YEAST CELLS STAINED WITH METHYLENE BLUE-EOSIN TECHNIQUE (100 CELLS SELECTED AT RANDOM).

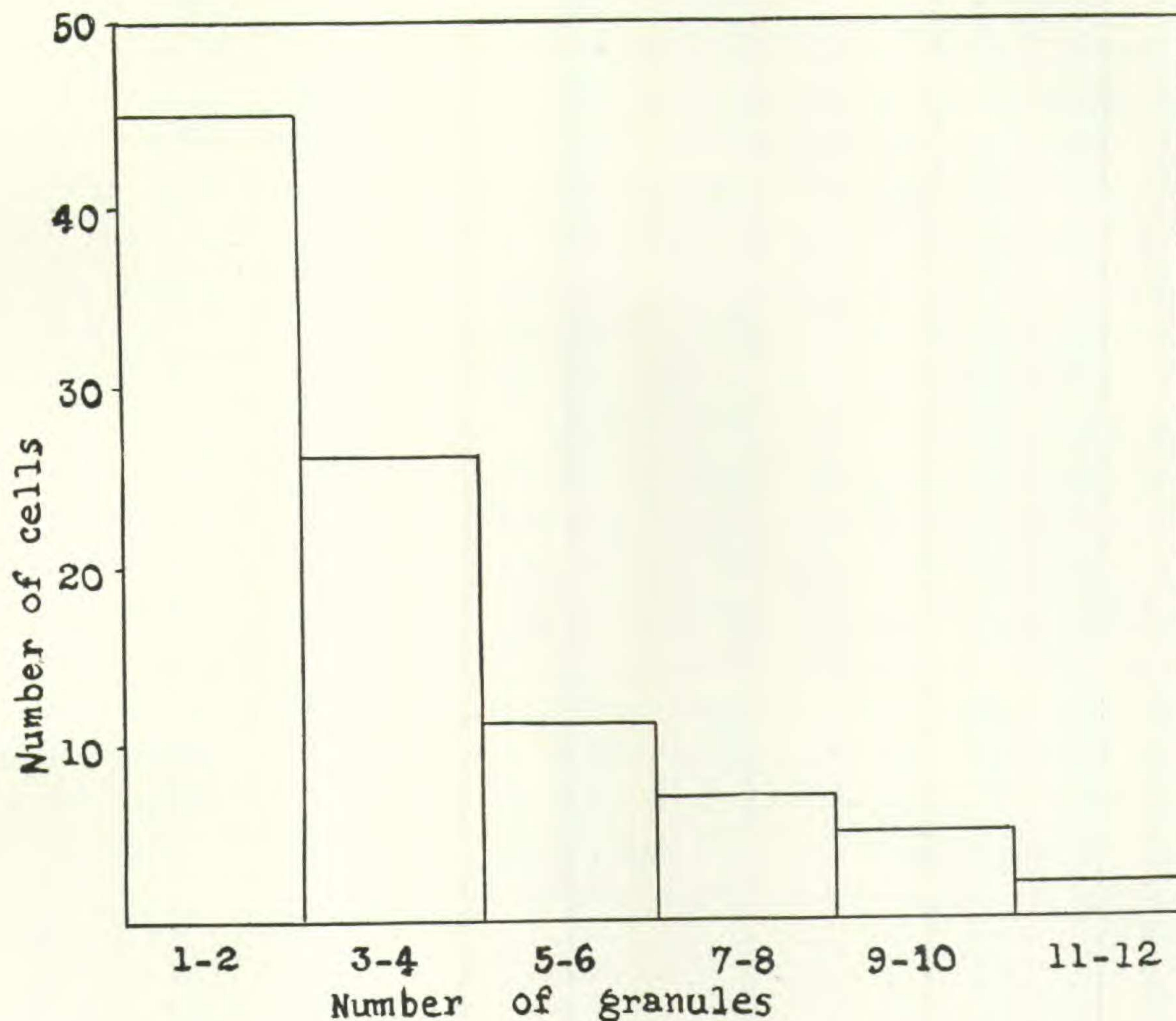
Number of particles per cell	0	1	2	3	4	5	6	7	8	9	10 or more
Number of cells	85	4	2	2	2	—	1	1	—	1	2

particles were exceedingly numerous and were present in most cells where differentiation was not carried too far. The parvicorp was not visible in these cells.

Granule number per cell was counted in 100 granule-containing cells (15 per cent of the total cell number). The results are diagrammed in text-fig. 2. Most of the cells in this culture contained few particles which at times appeared irregular in outline as if they were possibly aggregates, but the total amount of material in the magnicorps was not uniform.

In a few instances the particles appeared paired (Table V). The pairs of granules were similar in staining reaction, size, and shape. At times they occurred as separate particles; at times the two were joined in dumbbell ("diplokokken ähnlich", Henneberg, '16) or V-shaped masses with the two ends alike in shape and size. Rarely all of the particles of a cell appeared paired (pl. 14, fig. 107);

more often there were only one or two such pairs, the other particles not showing this condition. The number of pairs of granules was not constant. Due to the position and arrangement of granules in some cells it was not always possible to be certain whether or not they were paired. These are listed in Table V as "Possibly paired."



Text-fig. 2. Number of granules per cell in the magnicorps of 100 granule-containing cells.

TABLE V

PER CENT OF YEAST CELLS SHOWING PAIRING OF THE PARTICLES IN THE MAGNICORP WHEN STAINED WITH METHYLENE BLUE-EOSIN.

	% of granule-containing cells	% of total number of cells in culture
No apparent pairing	82.+	12.+
One or more pairs present	9.—	1.
Possibly paired	9.	1.+

Actively budding yeast from a yield test (4 hour 20 minute shaken culture) showed very faintly differentiated parvicorps and a few cells which contained as many as thirty to fifty stained particles. The latter were in or at the periphery of the magnicorp (pl. 14, fig. 108) and in several instances were passing into the bud. Due to the large number of somewhat similar granules in the cells pairing could not be determined.

Aceto-Carmine and Related Smear Techniques.—As mentioned in Table III, various concentrations and mixtures of acetic acid, lactic acid, and propionic acid were tried with the following dyes used singly and in combination: carmine, orcein, and Bismark brown. Results were generally unsatisfactory. Granular cytoplasmic material stained so deeply as to obscure the parvicorp in most cases and the magnicorp was generally shrunken and distorted. Various fixations beside acetic-alcohol and a number of other treatments and mordants suggested in the literature (Darlington and La Cour, '42) were tried with little success. Aceto-orcein and propionic carmine were somewhat more satisfactory than other solutions. Gentle heating helped bring out contrast, and the slides improved a little after standing for two to five days at room temperature. The cells which stained most clearly were those from the edge of giant colonies two to four weeks old, grown on nutrient agar in a moist chamber (pl. 14, figs. 113–119). Probably this is due to the relatively small amount of granular material in the cytoplasm of these cells (author's research, unpublished). The parvicorp may be rounded, or, more frequently, somewhat star-shaped, or may occasionally appear bipartite, especially in dividing cells. The magnicorp (if visible at all) is usually shrunken, distorted, and empty. Although much time was spent in trying to adapt this technique to yeast, it was finally abandoned as unsatisfactory.

Studies of Sporulating Cultures

Feulgen and Giemsa Techniques.—Because of the similarity of results with these techniques, they are considered together. In any sporulating culture only a few cells were in meiotic division at any one time, and the extremely small number found in certain stages would indicate that at least parts of the process are very rapid. Preceding spore formation, the parvicorp becomes approximately twice its normal diameter, appears filamentous, and resembles the prophase of the conventional meiotic division as closely as could be expected in anything as small and unique as a yeast cell (pl. 14, figs. 123–127; pl. 15, figs. 128–130, 141–144). This phase appears to be of long duration as it is quite common in slides of sporulating cultures. The next steps in the division are not too certain and probably proceed with great rapidity as few instances were found that could be definitely assigned to this phase in the many slides that were examined. Probably such stages as pl. 15, figs. 131, 145, 146, 149, 150 belong in this category. Very

rarely two parvicorps lying beside or above each other were found toward the center of the cell (pl. 15, fig. 151; pl. 16, figs. 173, 174). While this position might be the logical expectation, its extreme rarity leads one to believe that the second division normally follows the first so rapidly that the stage with two parvicorps may not actually become organized in most cells. The second division appeared to start near the center of the cell. Two elongate, slightly dumbbell-shaped masses were formed, the ends of which passed toward the periphery of the cell, usually toward opposite poles. A gradual thickening of the extremities of the masses occurred at the expense of the center (pl. 15, figs. 132–136; pl. 16, figs. 152–158). Such division figures were relatively common. Cells with elongate parvicorps which appeared bipartite or possibly four-partite were also relatively common in all sporulating material (pl. 15, figs. 147, 148). These may represent a stage which either follows the more diffuse early stage or might be the beginning of the second division. Instances of a single parvicorp at each pole of the cell were not positively ascertained; careful study of apparent examples usually showed that at least at one pole one body could be resolved above the other, and that the second division had actually taken place. When division is first completed, the parvicorps usually lie at the periphery of the cell close to the wall and the spore plasm lying between them is frequently more dense than the epiplasm[1] (pl. 16, fig. 160). The organization of the ascospores seems to occur in a manner similar to that of other Ascomycetes. Apparently the spore is delimited by being cut out by rays extending from the parvicorp. At times one or two of the ascospores develop more rapidly than the others. Ray-like strands of cytoplasm frequently extend out from the parvicorp in the fully formed ascospore. If only two or three ascospores develop, the other parvicorps can be observed lying free in the ascus (pl. 15, figs. 139, 140). Single spores with two or more parvicorps were not seen. Old agar slants of diploid cells, some sporulating, showed aberrant divisions in which the parvicorp apparently divided without subsequent cell division. In some of these cells the parvicorp was divided into separate particles (pl. 13, fig. 97).

Meiotic division of the magnicorp could not be followed in either technique. Although this body is generally visible in budding material stained with Feulgen, it was imperfectly visible, if at all, in sporulating cells (except in the fully developed ascospores where it again appeared empty).

Notes On Other Techniques.—Aceto-orcein was unsuccessful as a stain for the division figures of spore formation because of the deeply staining, granular cytoplasm. However, the formation of the ascospores was similar to that observed with Giemsa and in the fully developed ascospores an empty magnicorp was generally visible (pl. 17, figs. 193, 194).

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[1] In Ascomycetes, the cytoplasmic contents of the ascus not used in spore formation.

Methylene blue-eosin was not especially useful as a stain for sporulating material, nor did the conventional volutin reaction give an enlightening picture of the magnicorp and its particles in meiosis. With both techniques, if magnicorps were visible, stained particles in them were few (exceptionally there were many particles or none). No regular organization of magnicorp and its contents was ascertained.

Slides stained by the longer procedure for Heidenhain's hematoxylin (5a, Table III) or by Brazilin gave much the same results as Giemsa and Feulgen, but less distinct due to the retention of stain by the granular cytoplasm (pl. 16, figs. 165–175). If stained by the shorter method (5b, Table III), the results were so variable as to be uninterpretable and the companion body could not be followed. The magnicorp, though evident in budding and resting cells with these techniques, could not be followed in sporulating material.

Studies of Living and Supra-vitally Stained Cultures

Comparative studies on living cells of cultures were made whenever slides were prepared. In budding cells the parvicorp was only rarely visible in living cells, although its position could often be determined by a slight invagination of the magnicorp. The latter body could almost always be seen except in certain small, very dense cells where it was probably obscured by the highly refractive surrounding cytoplasm. It occurred usually as one body, occasionally as two, more rarely as several to many, depending on the age, nutrition, aeration, and other cultural conditions of the cells. At times one to several particles could be seen moving in the magnicorp, and occasionally these appeared to be paired.

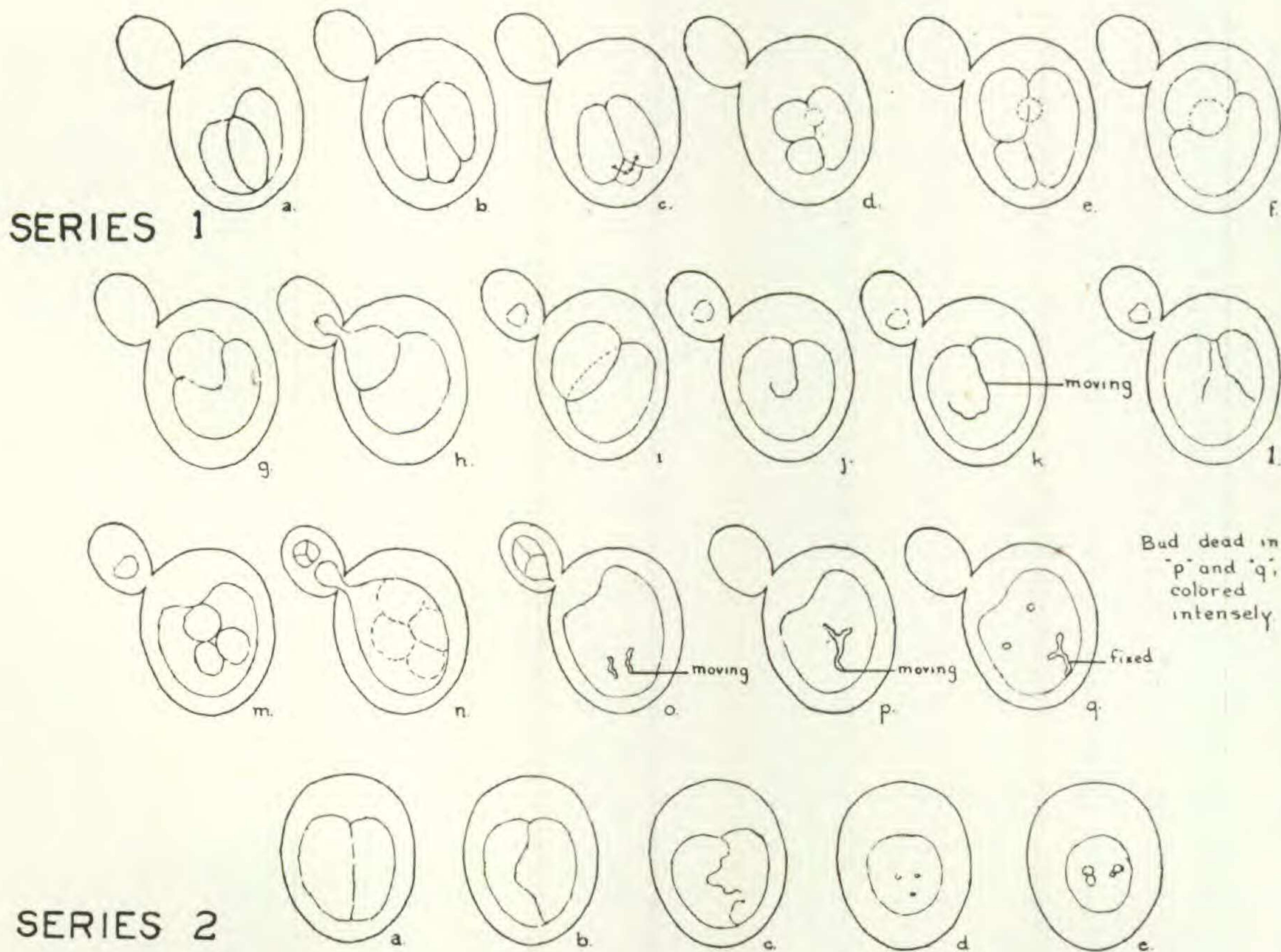
The cytoplasm of many living cells from presporulating cultures was so highly granular that all other structures were obscured. It was from these that spores developed. Although a number of methods of observing the formation of spores in living cells was tried, only the one described below was found satisfactory. Nothing externally visible occurred in the living cell the first twelve to sixteen hours on the gypsum slant, the variation in time depending upon the culture, temperature, presporulating medium, etc. After the culture had been on the gypsum slant for twelve hours samples were examined at half-hour intervals. The movements of yeast cells are sufficiently rapid that for accurate continued observation it was found imperative to restrain them as much as possible. The following technique prevented excessive motion, yet kept the yeast in viable condition under the microscope for five to seven hours. A tiny frayed-out fragment of lens paper was placed on a No. 1 cover glass and both were dipped in alcohol and flamed. With a little practice the lens paper fibers spread flat and evenly and are not charred. A drop of sterile water acidified to about pH 4 with acetic acid was placed on the lens paper and a few cells from the gypsum slant were added with a needle. The cover glass was placed cell-side down over a concave (drop) slide to provide an air chamber and the edges were sealed with wax. The

yeast cells gathered along the lens paper fibers, and Brownian movement was thus greatly reduced. Where the cells were not crowded, an observation every fifteen to twenty minutes kept a given cell or group of cells in view. Thus individual cells could be watched from the first sign of spore delimitation to complete spore formation, a process that took three to five hours at room temperature under these conditions. In acidified water with no air space, in agar, or in oil, sporulation also occurred but only sporadically and after one to five days.

In living cells the spores appeared to be delimited in the same manner as described for prepared slides. Several instances were observed in which the four spores were formed from the protoplasm at one end of a large cell. The other end was occupied by a body which resembled the magnicorps of other cells on the slide although no proof of its actual identity therewith was ascertained. A cell of this kind from a Giemsa-stained slide is illustrated in pl. 17, fig. 195. Generally the magnicorp was not visible in the living cells during the process of sporulation.

When iodine was applied to budding cells, it had the effect of temporarily bringing the parvicorp into "relief" and of emphasizing the particles in the magnicorp, often revealing them in rapid Brownian motion.

Toluidine blue was also applied to budding cultures. This dye at times caused



Text-fig. 3. Series of sketches of two cells under the influence of toluidine blue solution.

very striking reactions in the magnicorp, but the parvicorp was never visible. Changes in the magnicorp were especially apparent with continuous observation of a single cell. Text-fig. 3, Series 1, shows sketches of the changes in the magnicorp of a budding cell observed continuously for forty-five minutes. In "h" and "n" the magnicorp was seen to burst rapidly out into the bud. Series 2 shows a similar non-budding cell. Granules of various sizes often stain in the magnicorp with this procedure; occasionally also parts of the cytoplasm stain, and the cells finally die and become deeply colored throughout.

WILD YEASTS

Studies of Budding Cultures.—Several yeasts were collected in the wild and a few cytological observations were made on two of them. A brief account is given here of these fragmentary observations since they are rather suggestive when compared with the results with *Saccharomyces cerevisiae.* Had these yeasts been obtained earlier in the progress of the investigation more detailed observations on them would have been made.

The yeast represented in pl. 17 is one of a number which was isolated from wild fruit gathered late in the fall of 1945 at the Missouri Botanical Garden Arboretum near Gray Summit, Missouri. While it has not been positively identified, since it has not yet been induced to sporulate, it can be placed tentatively in the Fungi Imperfecti, possibly in *Syringospora* or *Blastodendrion* or a closely related genus and probably represents an undescribed species. It is an especially large-celled yeast that produces from two to twelve easily detached buds in bipolar position when growing actively on nutrient agar. A few Giemsa and Feulgen slides were made in order to study its cell organization when producing the higher number of buds.

From one to twelve parvicorps (Feulgen and Giemsa positive) were found per cell, the number depending on their size and the amount of budding. Commonly there were more parvicorps than buds. As was noted in the microscopic study of the living cells, the buds were very delicately attached and some of them may have been broken off in handling. Probably also the division precedes the bud formation in this species. While no attempt was made to check this fact, the very large number of parvicorps in certain cells (pl. 17, figs. 176, 177, 186) would suggest such a possibility. However, small, detached, immature buds without parvicorps were frequently observed. When numerous parvicorps were present they were often of two distinct sizes, the smaller ones staining more intensely. In contrast to *Saccharomyces cerevisiae* the parvicorp is often separated into particulate units at division (pl. 17, figs. 178, 180, 183, 187, 189). When several parvicorps were present all seemed to divide synchronously, as cells were noted with two, four, or eight in division at the same time. Dividing and non-dividing parvicorps were not observed in the same cell. Wherever the magnicorp

was visible it was empty, but when many parvicorps were present they sometimes seemed to fill the whole cell.

Another of the wild yeasts from the same collection (a *Cryptococcus*?) should be mentioned because under certain cultural conditions (usually when several days to a week old) moving particles in the magnicorp were the rule rather than the exception and many of the particles seemed paired in dumbbell fashion. In some sections of one particular slide almost half the magnicorps had two equal particles, closely paired, each pair moving as a unit. Under other conditions, especially in young or older cultures, this phenomenon was not apparent.

Discussion

The very nature of vegetative reproduction in budding yeasts would lead one to expect their mitoses and nuclear organization to exhibit certain exceptional phenomena. As there is nothing quite comparable to this budding process in the plant or animal kingdom, interpretation of nuclear organization should take into account as inclusive a picture of all cell components as possible. Both parvicorp and magnicorp seem to be associated with vegetative cell division and a complete interpretation must take cognizance of both. From genetics there is now exact indirect evidence on the organization of the yeast cell. The excellent work of Lindegren, Spiegelman, and Lindegren ('44) and of Winge ('39, '44) has demonstrated the regular Mendelian segregation of characters at meiosis. Diploid strains, reproducing only vegetatively, are stable and retain their cultural characteristics as would be expected; haploid cultures exhibit a far greater mutation rate. This is easily observed in giant colony structures where haploids show frequent sector mutations which are generally lacking in the more stable diploids. Therefore in spite of its small size and peculiar vegetative reproduction yeast cannot be greatly different in its fundamental organization from other organisms although in various superficial details of mitosis and meiosis one may confidently expect considerable modification.

Similar superficial modifications of mitosis and meiosis have been reported for a variety of tissues and organisms. In the pollen tube of the spermatophytes, when mitosis of the generative cell must occur within narrow confines, it is often somewhat atypical; the metaphase plate may be greatly elongated and there may be no visible achromatic figure (Trankowski, '30). In the Protista, Belar ('26, '28) has shown that fundamentally regular but superficially atypical nuclear organization is not uncommon. As mentioned before, several conflicting interpretations of nuclear organization of yeast have been presented in the past and there is to date no general agreement. Table VI presents in tabular form the varying interpretations by previous authors of the disputed entities of the yeast cell.

In the light of the work of Mazia and Jaeger ('39), Caspersson and Schultz ('38), Caspersson ('39a, '39b, '40), Mirsky ('43), Mirsky and Pollister ('43a, '43b), Pollister and Mirsky ('43, '44), Greenstein ('44), Davidson and Way-

mouth ('44), and many others, the Feulgen positive staining of the parvicorp would seem indicative of its nuclear nature. The constancy of the parvicorp as a cell component and its non-homogeneous character when stained with Giemsa supports this interpretation. Opponents to this view point out that the division of the parvicorp appears to be amitotic. Since division is intra-nuclear in many fungi, this fact plus the very small size of the parvicorp make resolution of individual chromosomes difficult with the ordinary microscope. In the wild yeast described above particulate units were observed at certain division stages in both Feulgen and Giemsa slides. In the early stages of meiotic division in S. *cerevisiae* the parvicorp becomes enlarged and appears filamentous, resembling the usual early meiotic prophase nucleus, but its small size makes accurate resolution difficult. As is true of many nuclei, the parvicorp stains more intensely with Feulgen at some stages than it does at others. It is visible in all stages of division in all cells stained with Feulgen and Giemsa and with Heidenhain's hematoxylin and Brazilin if destaining is not carried too far, but is rarely visible in the living cells. In ultra-violet photographs (Caspersson and Brandt, '41; Brandt, '41) the two types of nucleic acid are indistinguishable. These authors state that this technique does not generally differentiate the nucleus in yeast. In the very actively budding cells, however, the parvicorps appear in the photographs as diffuse, somewhat lighter areas in the deeply absorbing cytoplasm, sometimes seeming not much more absorbent than the magnicorp which they state contains very little nucleic acid.

The magnicorp enters the bud before the parvicorp and is probably of importance in bud initiation as suggested by Lindegren ('45). If particles are visible within the magnicorp they also enter the bud, and at times they seem to be paired or assume form or position difficult to explain as "reserve stuff." These phenomena plus the apparent amitosis of the parvicorp have led to the interpretation (Janssens and Leblanc, '98; Wager and Peniston, '10; Lindegren, '45) of the magnicorp and its contents as the nuclear vacuole and chromatin respectively. Although the magnicorp and its contents are Feulgen negative at all stages of growth and division, Lindegren ('45) has pointed out that the desoxyribosenucleoprotein nature (Feulgen positive) of nuclei of higher organisms does not necessarily indicate its universality in the chromatin of simpler organisms. However, if the magnicorp is interpreted as the nuclear vacuole and the parvicorp as the centriole, the latter is not usually Feulgen positive. If visible, the particles in the magnicorp vary greatly as to size, shape, and number: (a) with physiological conditions—age, nutrition, aeration, etc.; (b) with the strain; and (c) with fixation. Their chemical composition is not known with certainty, but common nuclear fixing solutions are not generally effective, and special fixation, as, for example, with formaldehyde, must usually be employed. As pointed out earlier, the magnicorp is usually a single body, but often seems to occur as two bodies, one at either end of the parvicorp. In old or starved cultures it may appear to be divided into a number of parts (Brandt, '41), in which case the particles may occur in any or

all the parts of the magnicorp. Budding cells very commonly show no stained particles with the techniques employed. The number of particles counted in cells of different cultures stained with methylene blue-eosin varied from none in most cells to as high as fifty. Van Herwerden ('18) found the number of particles related to phosphorus content of the nutrient medium. With dark field illumination the rate of Brownian motion of particles in the magnicorp and in the surrounding medium is similar, indicating a very low viscosity. On the contrary, the lack of Brownian motion in the vicinity of the parvicorp and its tendency to bulge into the magnicorp would indicate a higher viscosity for the former. The pressure changes of the magnicorp with toluidine blue are likewise indicative of low viscosity, a character more usually associated with vacuolar behavior than with nuclear "sap." Difficulty in following any organization of the magnicorp through meiotic division in this investigation (also by Janssens and Leblanc, '98; and Wager and Peniston, '10) points strongly away from a nuclear interpretation but improved techniques may alter this. However, the presence of the magnicorp in the fully developed spore indicates its importance as a cell entity.

Various chromosome numbers in various parts of the cell have been suggested: two—both haploid and diploid—by Badian ('38); approximately eight—diploid —by Kater ('27) in the parvicorp; and twelve by Lindegren ('45) in the magnicorp. Although a number of investigators have noted the division of the parvicorp into two bodies at times and have called these chromosomes, present genetic evidence (Lindegren, personal communication, unpublished) does not point to this chromosome number, and cytological evidence is as yet uncertain. Undoubtedly the parvicorp is divided into two bodies at times, but if these are chromosomes they are large enough that they should be seen occasionally in metaphase or anaphase configuration. If the parvicorp is nuclear, further refinement of technique is necessary to be certain of chromosome number. If the magnicorp is nuclear, the variability of the number of particulate units is suspiciously great. However, because of the small size of the yeast cell and its lability under various cultural conditions, this objection has less force than it would have in other material. As mentioned previously, the parvicorp of the wild yeast described above appeared to separate into particulate units during mitosis but these were too crowded together for the number to be certain.

At least four parts of the yeast cell have been interpreted as the nucleolus (Table VI). Wager and Peniston ('10) considered the parvicorp as the nucleolus with the chromatin network extending from it over the magnicorp. This places the "nucleolus" entirely outside of the "nucleus" as they interpreted it, certainly an unusual position for it. Brandt ('41) and Caspersson and Brandt ('41) consider the ribonucleic acid-containing granules scattered throughout the cytoplasm (designated as "volutin" by them) the equivalent of the heterochromatin and nucleolus of higher organisms. Janssens and Leblanc ('98) sometimes called the central granule of the magnicorp the nucleolus, and Guilliermond ('20) and

TABLE VI

INTERPRETATIONS OF DISPUTED ENTITIES OF CELL ORGANIZATION BY VARIOUS INVESTIGATORS OF YEAST CYTOLOGY

Cell entity	Janssens and Leblanc, '98	Guilliermond, '03, '10, '20	Kohl, '08	Wager and Peniston, '10	Henneberg, '16	Kater, '27	Winge and Laustsen, '35, '37	Badian, '37	Beams et al., '39	Brandt, '41	Lindegren, '45	Subramaniam and Ranganathan, '45, '46a, '46b
Parvicorp	Part of nucleus (sometimes nucleolus?)	Nucleus containing nucleolus	Nucleus, possibly containing nucleolus	Nucleolus	Nucleus	Nucleus containing nucleolus (approx. 8 chromosomes)	Nucleus—probably 2 chromosomes	Nucleus—2 chromosomes in both haploid and diploid	Nucleus	Euchromatin of nucleus (2 chromosome equivalents)	Centriole (bipartite)	Nucleus—normally of 2 equal chromosomes
Magnicorp	Part of nucleus	Vacuole	Vacuole	Nuclear vacuole	Vacuole	Vacuole	Vacuole	Vacuole	Vacuole	Vacuole	Nuclear vacuole	——
Particles in magnicorp (may or may not be in motion)	?	Metachromatin (volutin)	Volutin	Chromatin network	Volutin	*No* particles in magnicorp	——	Volutin	——	Metachromatic granules	6 pairs of chromosomes	——

TABLE VI (continued)

Refractive, non-fatty cytoplasmic granules	?	Basophile granules	Volutin? and "Eiweiss-krystalle"	Volutin	Volutin?	Metachromatic granules (often a reticulum extending out into cytoplasm from parvicorp)	—	Volutin	—	Volutin—equivalent of heterochromatin and nucleolus of higher organisms also metachromatin	—	—
Companion body	?	—	—	Chromatin patch	—	—	—	—	—	—	Mentioned	—
Central granule of magnicorp (in motion)	Nucleolus?	—	—	Central volutin granule	—	—	—	—	—	—	Possibly "balled up" chromosomes	—

Kater ('27) thus designated the deeper-staining body or bodies in the parvicorp. At times one or more such bodies are visible in the Giemsa-stained slides, but whether these are sufficiently consistent to be considered the nucleoli is questionable.

Whether or not a membrane surrounds the parvicorp has been a question of debate. Its presence is indicated by the regularity of outline of the parvicorp after certain treatments, for example, with methylene blue-eosin and with Heidenhain's hematoxylin (short procedure; pl. 13, figs. 82–88; pl. 14, figs. 98–108). It is true that the parvicorps in Giemsa and Feulgen slides often show an irregular outline. Acid hydrolysis has been carried out with both of these, and although Bauer ('32) has demonstrated that this treatment does not alter the structure of the desoxyribosenucleoprotein-containing material, there is no reason to believe that a membrane, if present, would stain. Studies of the magnicorp indicate that there is a membrane separating it from the cytoplasm. At times it appears to be a double membrane for occasionally after fixation the magnicorp seems to shrink away from the cytoplasm in places as if both possessed membranes.

If the nucleo/cytoplasmic ratio is considered, the parvicorp alone gives a ratio which is possibly slightly low, the combined parvicorp and magnicorp an exceptionally high one.

It is thus apparent that after one hundred years of cytological work, the organization of the yeast nucleus is still a matter for debate among authorities, even as to the most elementary points. Direct observation, uncorrelated with other techniques, must probably continue to be relatively unproductive with objects as microscopically refractory as the yeast cell. However, direct cytological observation in combination with other techniques presents a more encouraging picture. Among the most promising of these developments are the following:

(1) Additional genetic studies such as those of Lindegren and Winge. Eventually they should demonstrate the chromosome numbers, their comparative size, and even chiasma frequencies and positions.

(2) Further biochemical studies along the lines of those of Mirsky and Pollister on higher organisms, combined with studies of the effects of enzymatic action on the several cell entities. A more complete knowledge of the chemical nature of the particles in the magnicorp and of the refractive granules in the cytoplasm should aid in an understanding of their role in cell organization and metabolism.

(3) Comparative cytological studies of wild yeasts. Species with larger cells or clearer cytoplasm should reveal more details of cell structure. Wild yeasts which reproduce by budding but which also develop true or pseudo-mycelia are frequently encountered. A cytological study of these species would relate the recognized entities of the yeast cell to the more usual mycelial type of growth.

(4) Additional investigations with ultra-violet and "phase-difference" microscopes and the study of thin sections with the electron microscope.

(5) Further refinements of cytological techniques. A method of removing, softening, or breaking the firm cell wall (possibly by enzymatic digestion, chem-

ical maceration, or pressure) which would permit spreading of the cell contents without excessive distortion should make further details of the parvi- and magnicorp resolvable. A study of the effects of cold treatment on different stages of spore formation may make it possible to obtain many more cells at one time in certain phases of meiosis. New techniques, as well as improvements in present fixation and staining procedures, should provide additional information for correlation with other methods.

Summary

1. A review of the literature on the cytology of yeasts showed great confusion in:

(1) use of terms,

(2) interpretation of nuclear structure.

(This is demonstrated in tabular form in Table VI.) An attempt was made to apply systematically a whole battery of techniques to both sporulating and budding material of yeast *(S. cerevisiae)*.

2. All of the techniques tried out are listed and described. Those used extensively are discussed in detail.

3. A descriptive terminology for the major cell entities (parvicorp, magnicorp, companion body, text-fig. 1, p. 254) is adopted in order to permit presentation of observations unbiased by earlier interpretations.

4. Fragmentary observations on two wild yeasts are included for comparison (pp. 264–265).

5. The "parvicorp" is a Feulgen positive, non-homogeneous, constant cell entity. Vegetative division does not appear to be typically mitotic in *S. cerevisiae*, but at least the prophase of meiosis resembles conventional configurations. A large-celled wild yeast showed particulate units of the parvicorp at mitosis.

6. The "magnicorp" (vacuole or nuclear vacuole of most authors) is Feulgen negative. It is almost universally present in budding material and enters the bud before the parvicorp, but is generally invisible in presporulating and sporulating material. Granules of the magnicorp are lacking in most cells with the techniques employed, but, when present, occasionally appear paired.

7. Relationships of these observations to the several interpretations of nuclear organization are discussed briefly (pp. 265–271), and suggestions are made as to possible future advances in this field.

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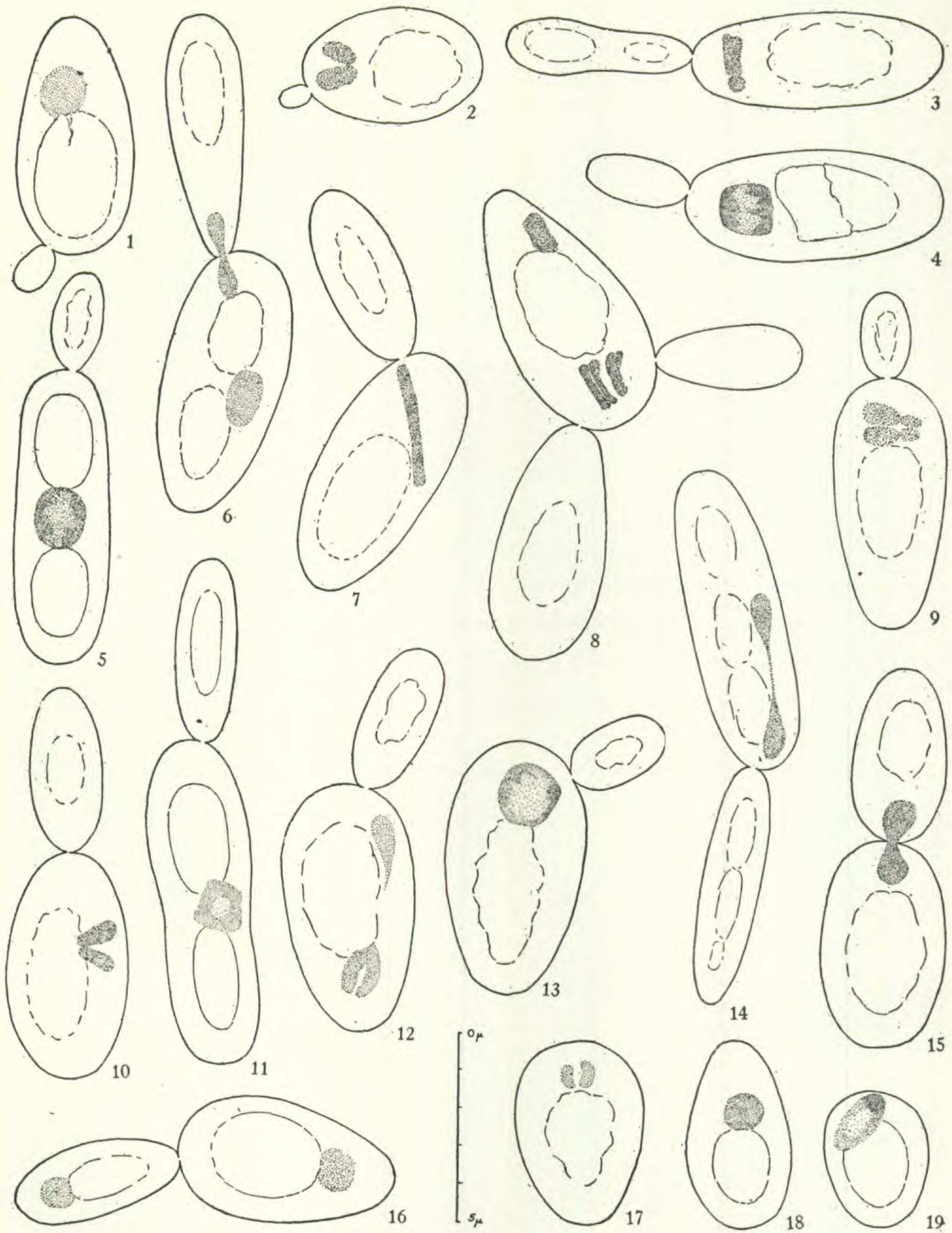
Explanation of Plate

PLATE 10

Cells from budding culture of *Saccharomyces cerevisiae* (Feulgen technique).

Figs. 1–19. Shaken broth culture, diploid (LK) strain, mercuric chloride fixation.



Budding material—Feulgen

NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

PLATE 11

Cells from budding cultures of S. *cerevisiae* (Robinow's Giemsa technique)—all 12–24-hr. broth cultures except fig. 46.

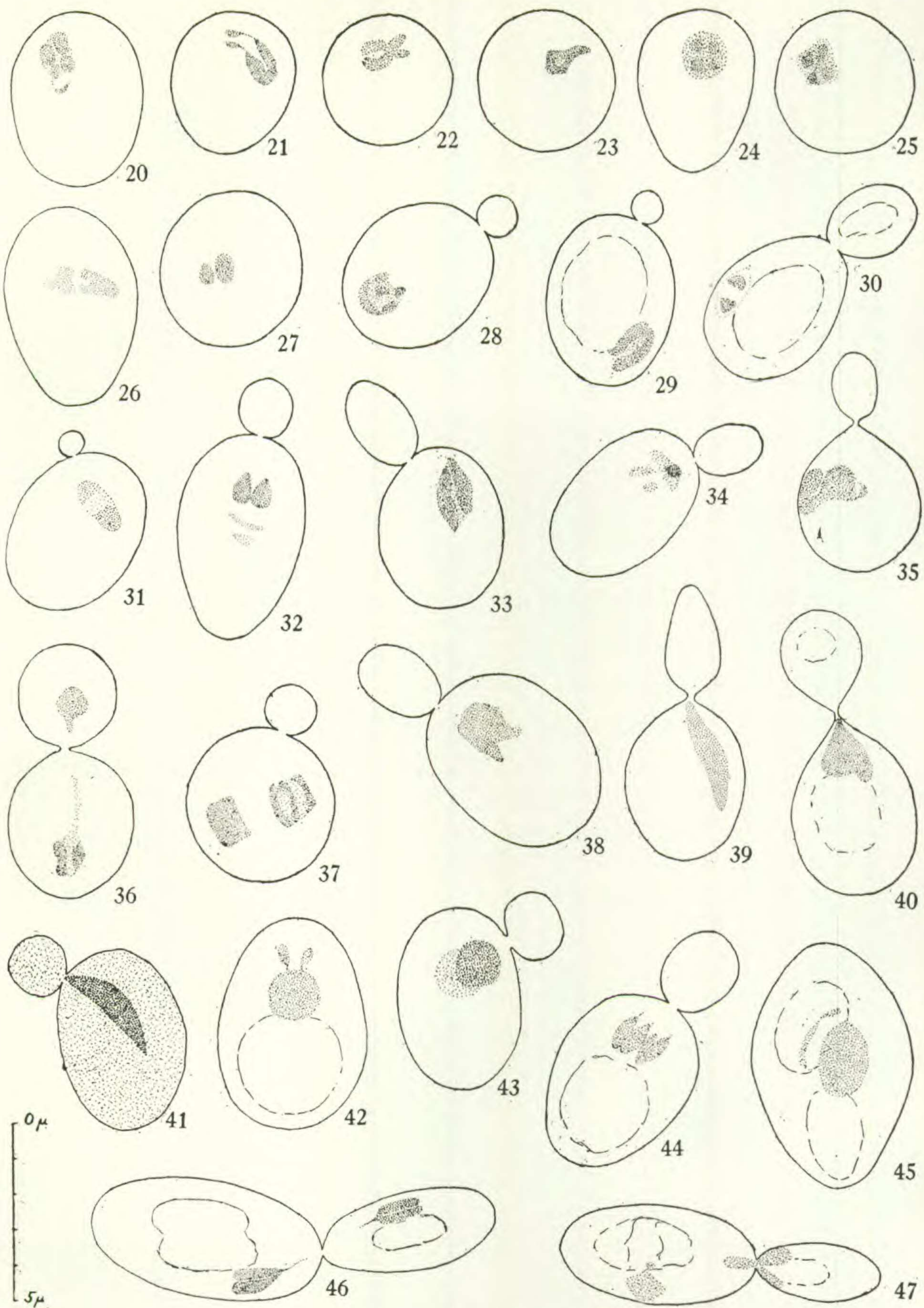
Figs. 20–41, 43. Haploid (Bc20—) strain.

Figs. 42, 44, 45, 47. Diploid strains.

Fig. 46. Budding cell from sporulating culture 24 hrs. on gypsum slant, diploid intra-specific hybrid.

Figs. 20–34, 38–42, 44, 45, 47. Iodine-glacial acetic acid-formalin fixation.

Figs. 35–37, 43, 46. Mercuric chloride fixation.



Budding material — Giemsa

NAGEL—SACCHAROMYCES CEREVISIAE

EXPLANATION OF PLATE

PLATE 12

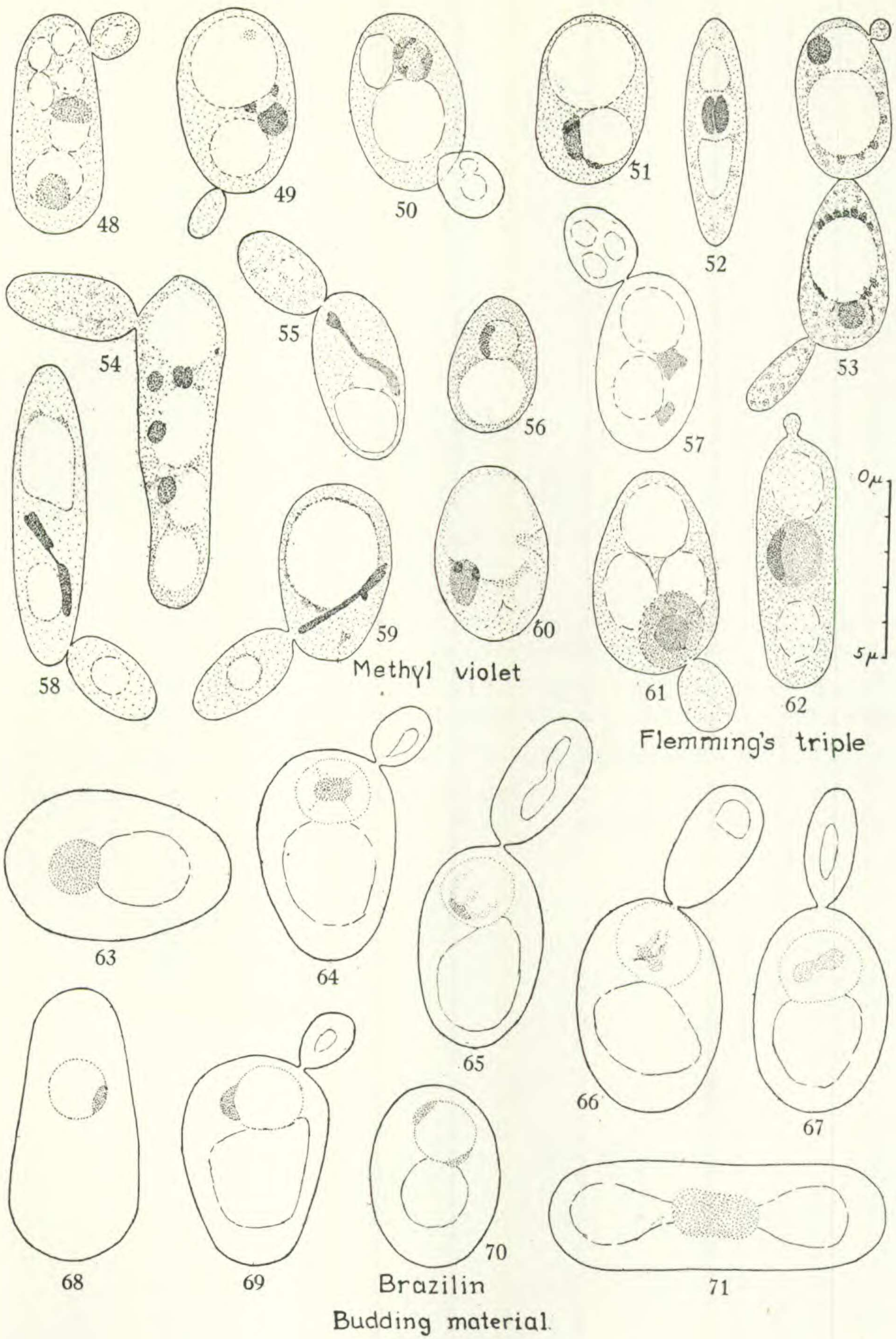
Cells from budding cultures of S. *cerevisiae* (methyl violet, Flemming's triple, or Brazilin technique).

Figs. 48–60. Johansen's methyl violet stain, broth culture, diploid (LK) strain, Navashin fixation (figures not drawn to scale).

Figs. 61, 62. Flemming's triple stain, otherwise as above.

Figs. 63, 68, 71. Brazilin (with short destaining), non-sporulating cells from gypsum slant, diploid (LK) strain, mercuric chloride fixation.

Figs. 64–67, 69, 70. Brazilin (with long destaining), broth culture from yield test, diploid intra-specific hybrid, mercuric chloride fixation.



NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

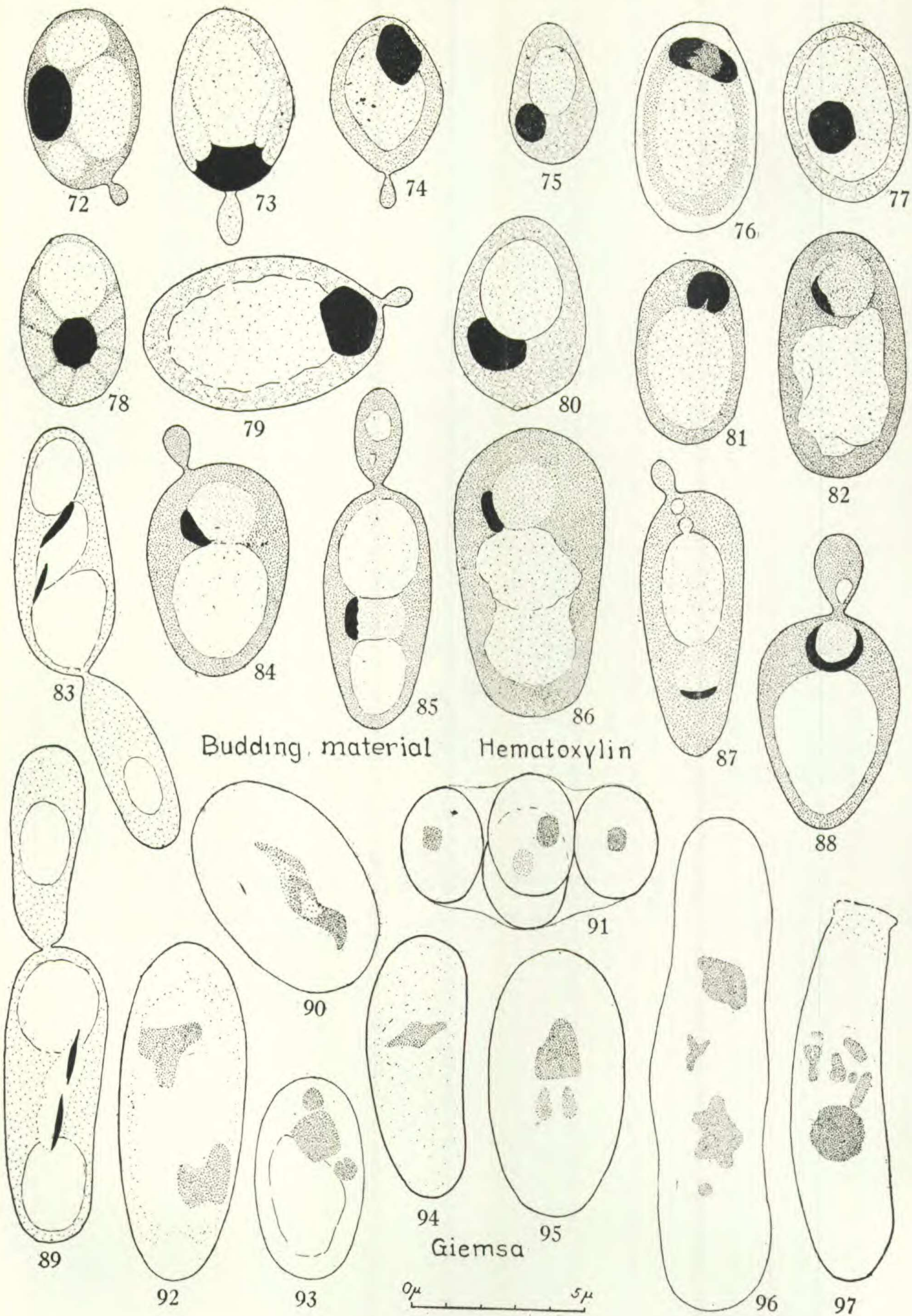
PLATE 13

Cells from budding culture of S. *cerevisiae* (Heidenhain's hematoxylin) and from agar slant (Robinow's Giemsa technique).

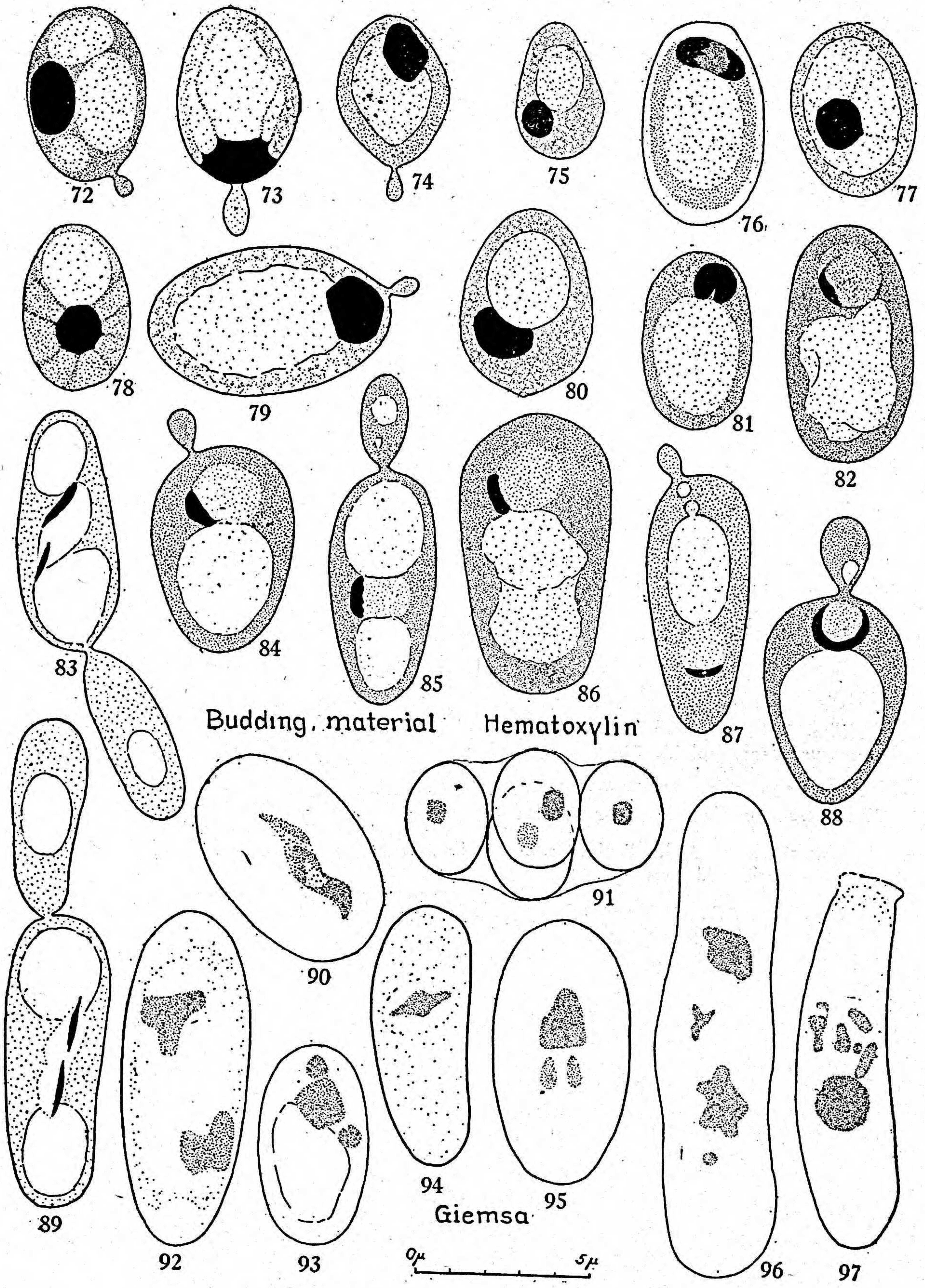
Figs. 72–81. Heidenhain's hematoxylin stain (5a, Table III), 24-hr., unshaken broth culture, diploid (LK) strain, mercuric chloride fixation.

Figs. 82–89. Heidenhain's hematoxylin stain (5b, Table III), otherwise as above.

Figs. 90–97. Robinow's Giemsa stain, 7-week-old nutrient agar slant, diploid (LK) strain, osmic acid vapor fixation.



NAGEL—SACCHAROMYCES CEREVISIAE



NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

PLATE 14

Cells from budding cultures of S. *cerevisiae* (methylene blue-eosin or aceto-orcein technique) and from sporulating cultures (Feulgen technique).

Figs. 98–107. Methylene blue-eosin stain, 48-hr., unshaken broth culture, diploid intra-specific hybrid, fixation in sat. picric acid in 70 per cent alcohol.

Fig. 108. Methylene blue-eosin stain; 4-hr. shaken yield test, broth culture, diploid, osmic acid vapor fixation.

Figs. 109–112. Same as figs. 98–107 except culture was shaken.

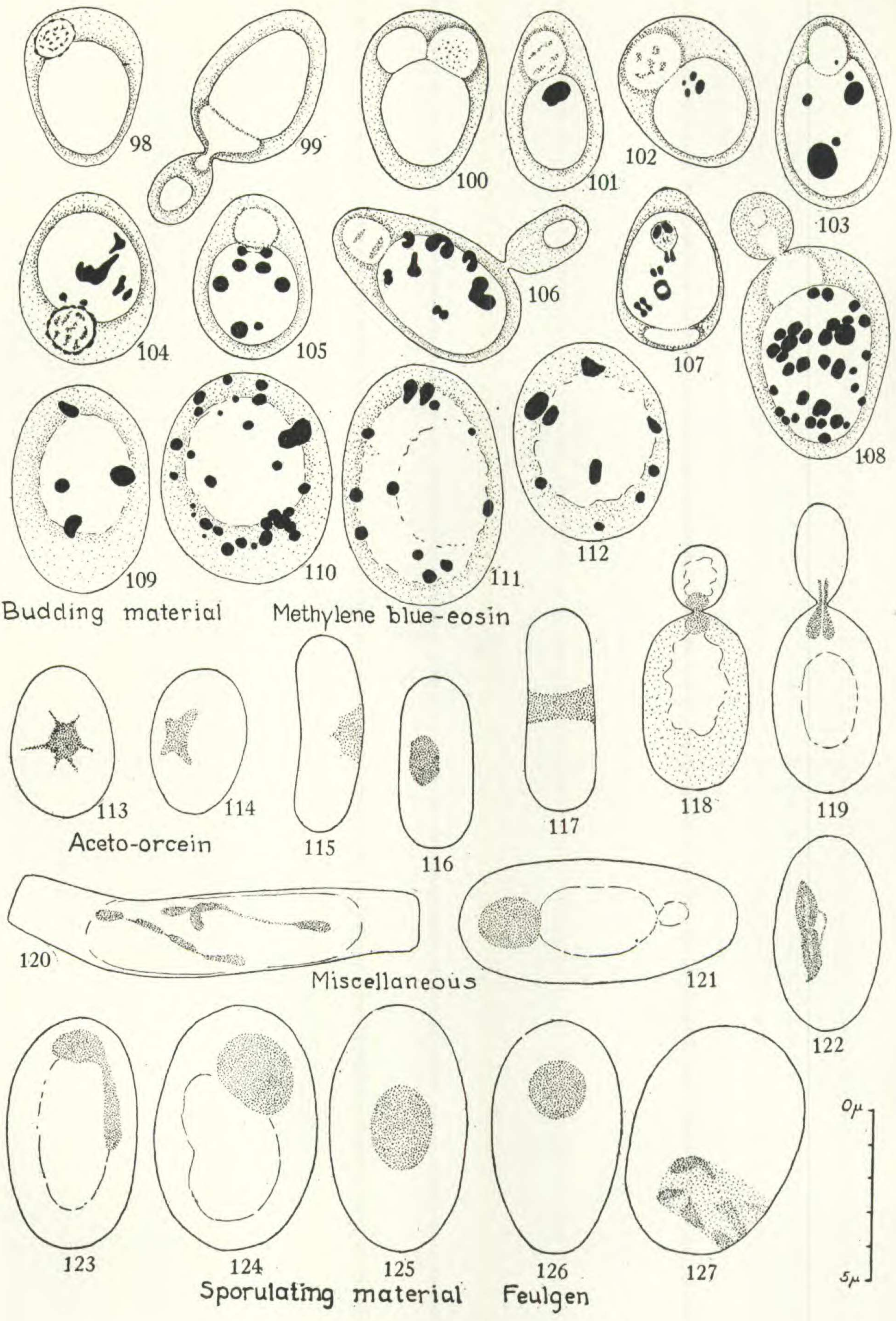
Figs. 113–117. Aceto-orcein stain, edge cells from giant colonies, various haploid strains.

Figs. 118, 119. Aceto-orcein stain, budding cells from sporulating culture 24 hrs. on gypsum slant, diploid (EFF) strain.

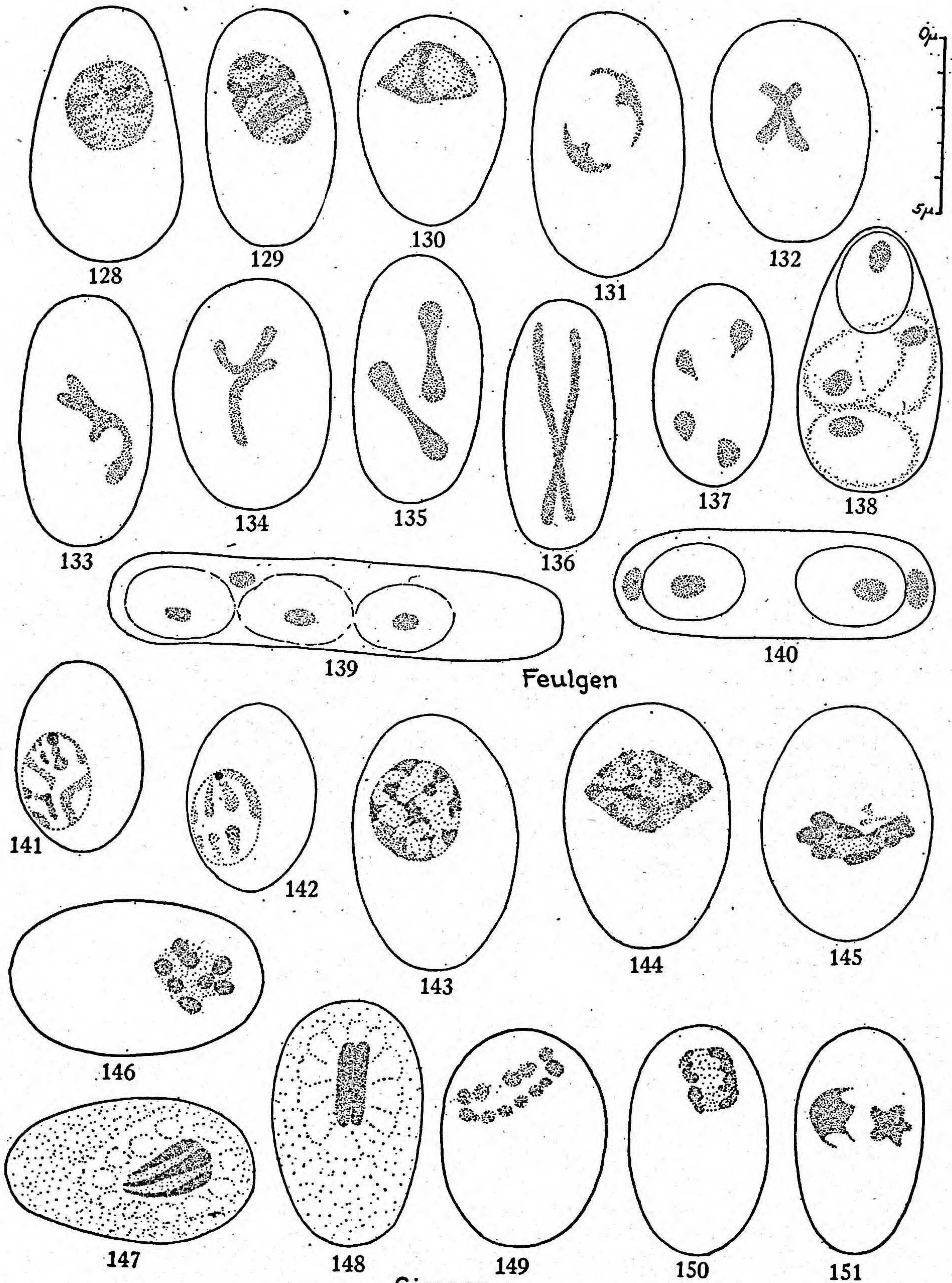
Figs. 120, 122. Robinow's Giemsa stain, week-old nutrient agar slant, diploid (EFF) strain, osmic acid vapor fixation.

Figs. 121, 124–126. Feulgen stain, 48 hrs. on presporulating medium, diploid (LK) strain, mercuric acid fixation.

Figs. 123, 127. Feulgen stain, sporulating culture 17 hrs. on gypsum slant, diploid (LK) strain, mercuric acid fixation.



NAGEL—SACCHAROMYCES CEREVISIAE



NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

PLATE 16

Cells from sporulating cultures of S. *cerevisiae* (Robinow's Giemsa, Heidenhain's hematoxylin, or Brazilin technique).

Figs. 152–164. Robinow's Giemsa stain, sporulating culture 24 hrs. on gypsum slant, diploid, mercuric chloride fixation.

Figs. 165–169. Heidenhain's hematoxylin stain (5a, Table III), sporulating culture 16 hrs. on gypsum slant, diploid (LK) strain, fixation in 48 per cent sat. aq. mercuric chloride, 2 per cent glacial acetic acid, 50 per cent picric acid.

Figs. 170–175. Brazilin stain, otherwise as above.

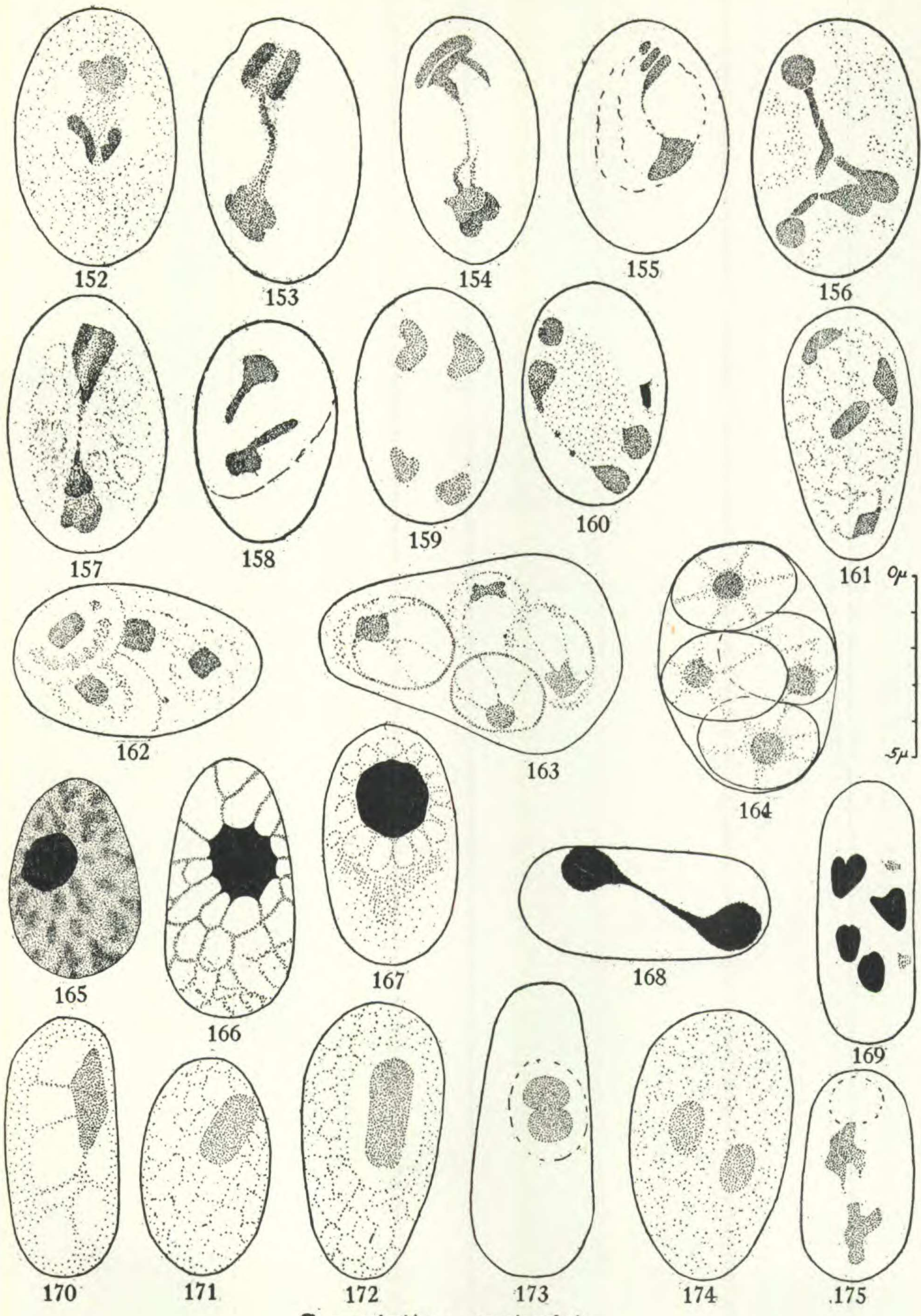
Explanation of Plate

PLATE 15

Cells from sporulating cultures of S. *cerevisiae* (Feulgen or Robinow's Giemsa technique).

Figs. 128–140. Feulgen stain, sporulating culture 17 hrs. on gypsum slant, diploid (LK) strain, mercuric chloride fixation.

Figs. 141–151. Robinow's Giemsa stain, sporulating culture 24 hrs. on gypsum slant, diploid, mercuric chloride fixation. Figs. 141, 142 are same cell shown at two levels (smaller scale than other figures).



Sporulating material

NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

PLATE 17

Budding cells of wild yeast (Robinow's Giemsa or Feulgen technique) and sporulating cells of S. *cerevisiae* (aceto-orcein or Robinow's Giemsa technique).

Figs. 176–190. Robinow's Giemsa stain, 24-hr. agar slant, wild yeast, osmic acid vapor fixation. In figs. 176, 177, 186 parvicorps are shown with solid outlines, magnicorps with dotted outlines.

Figs. 191, 192. Feulgen stain, otherwise as above.

Figs. 193, 194. Aceto-orcein stain, sporulating culture 24 hrs. on gypsum slant, diploid (EFF) strain.

Fig. 195. Robinow's Giemsa stain, sporulating culture 24 hrs. on gypsum slant, diploid, mercuric chloride fixation.